Attorney's Dock o.: 12875-002001 / 0643-5299US

Applicant: Wei-Yu Lo et Serial No.: 09/778,516
Filed: February 7, 2001

Page

2 of 8

In the claims:

Please amend the claims as follows:

1. (Currently Amended) A Lac shuttle vector, comprising:

(a) a region which regulates a plasmid copy number, wherein said region comprises an E. coli replication origin sequence;

(b) a eukaryotic gene expression cassette, which comprises a eukaryotic gene transcriptional promoter sequence, a multiple cloning site and a transcriptional terminator sequence, wherein a desired gene is inserted into said multiple cloning site;

(c) a lactic acid bacterial plasmid sequence, which comprises a plus origin of replication, and a nucleic acid sequence encoding a protein which is involved in replication of the lactic acid bacterial plasmid; and

- (d) a marker gene that is not an antibiotic resistance gene and <u>is operably linked to a</u> the promoter sequence thereof.
- 2. (Original) The Lac shuttle vector as claimed in claim 1, wherein said eukaryotic gene transcriptional promoter is cytomegalovirus (CMV) promoter.
- 3. (Currently Amended) The Lac shuttle vector as claimed in claim 1, wherein said lactic acid bacterial plasmid sequence is the plasmid of 2.1 kb in size isolated from Lactobacillus plantarum.
- 4. (Currently Amended) The Lac shuttle vector as claimed in claim 3, wherein the protein which is involved in the lactic acid bacterial plasmid replication is Rep A protein consisting essentially of 317 amino acids.
- 5. (Previously Amended) The Lac shuttle vector as claimed in claim 1, wherein said marker gene is β -galactosidase gene.
- 6. (Original) The Lac shuttle vector as claimed in claim 5, wherein the promoter of said β -galactosidase gene is erythromycin resistance gene promoter.

Attorney's Dock o.: 12875-002001 / 0643-5299US

Applicant: Wei-Yu Lo et Serial No.: 09/778,516
Filed: February 7, 2001

Page : 3 of 8

7. (Currently Amended) The Lac shuttle vector as claimed in claim 1, wherein the Lac Shuttle vector comprises the nucleotide sequence set forth in SEQ ID NO:1 or a complementary nucleotide sequence thereto, or a degenerate variant thereof <u>that contains</u> <u>degenerative protein-coding sequences</u>.



- 8. (Currently Amended) The Lac shuttle vector as claimed in claim 1, wherein the Lac Shuttle vector comprises the nucleotide sequence set forth in SEQ ID NO:2 or a complementary nucleotide sequence thereto, or a degenerate variant thereof <u>that contains</u> <u>degenerative protein-coding sequences</u>.
- 9. (Previously Amended) The Lac shuttle vector as claimed in claim 1, wherein the Lac shuttle vector is selected from the group consisting of:
- (a) pCLP7 having the configuration of restriction sites in FIG. 4, American Type Culture Collection Accession No. PTA-2661; and
- (b) pCLP8 having the configuration of restriction sites in FIG. 4, American Type Culture Collection Accession No. PTA-2663.
- 10. (Currently Amended) The Lac shuttle vector as claimed in claim 1, wherein the vector is for transforming a host cell, the host cell being transformed is a Gram-positive bacterium, and the endogenous β-galactosidase gene of the host cell is being non-functional.
- 11. (Previously Amended) The Lac shuttle vector as claimed in claim 10, wherein the host cell is the Lac- mutant of *Lactobacillus casei*, subsp. casei, which is designated Ana-1, American Type Culture Collection Accession No. PTA-2662.
 - 12. (Previously Amended) A kit for expression of a gene, comprising:
 - (a) the Lac shuttle vector as claimed in claim 1;

Applicant: Wei-Yu Lo et

Serial No.: 09/778,516 Filed

: February 7, 2001

Page

: 4 of 8

(b) a host cell in which the endogenous β-galactosidase gene thereof is non-functional; and

- a eukaryotic cell. (c)
- 13. (Currently Amended) A DNA vaccine composition comprising a Lac shuttle vector that contains:

Attorney's Dock

o.: 12875-002001 / 0643-5299US

- a region which regulates a plasmid copy number, wherein said region comprises (a) an E. coli replication origin sequence;
- a eukaryotic gene expression cassette, which comprises a eukaryotic gene (b) transcriptional promoter sequence, a multiple cloning site and a transcriptional terminator sequence, wherein an antigenic gene is inserted into said multiple cloning site;
- a lactic acid bacterial plasmid sequence, which comprises a plus origin of replication, and a nucleic acid sequence encoding a protein which is involved in replication of the lactic acid bacterial plasmid; and
- (d) a marker gene that is not an antibiotic resistance gene and is operably linked to a the promoter sequence thereof.
- 14. (Currently Amended) A method for selection of a host cell containing a vector, comprising:
- (i) introducing into said host cell the Lac shuttle vector as claimed in claim 1. wherein the endogenous β -galactosidase gene of said host cell is non-functional; and
- culturing said host cell transformed in step (i) under conditions in which lactose is (ii) the only carbon source, thereby selecting a host cell comprising the Lac shuttle vector of claim 1.